Helsinki University Central Hospital
Department of Otorhinolaryngology
University of Helsinki, Finland

PAEDIATRIC OTORHINOLARYNGOLOGICAL FINDINGS IN LUANDA, ANGOLA

Anni Taipale

ACADEMIC DISSERTATION

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Supervised by

Professor Anne Pitkäranta, MD, PhD
Helsinki University Central Hospital
Department of Otorhinolaryngology
University of Helsinki

Reviewed by

Docent Juha-Pekka Vasama, MD, PhD
Tampere University Central Hospital
Department of Otorhinolaryngology
University of Tampere

Docent Aino Ruohola, MD, PhD
Turku University Hospital
Department of Paediatrics
University of Turku

Official opponent

Professor Malou Hultcrantz, MD, PhD
Karolinska University Hospital
Ear, Nose and Throat Department
Karolinska Institute
Stockholm, Sweden

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1. CONTENTS

1. ABSTRACT

Background: Otorhinolaryngological (ORL) diseases and hearing loss frequently occur among the children of developing countries, but they remain poorly characterized. A need therefore exists for more comprehensive understanding of the background and clinical features of these diseases in resource-poor settings.

Patients and methods: Voluntary paediatric outpatients of various specialties were examined at Hospital Pediátrico in Luanda, Angola. Study participants underwent medical history-taking, a thorough physical and ORL examination, hearing screening by brainstem auditory-evoked potentials (BAEP), and, at age 5 or older, pure-tone audiometry. Nasopharyngeal smears (NPS) were obtained from 102 children with respiratory symptoms and screened by polymerase chain reaction for human rhino- (HRV) and enterovirus (HEV). We took 18 bacterial culture smears from children with chronic suppurative otitis media (CSOM). Clinical data collected were compared between 23 children with CSOM vs. 23 age- and gender-matched controls, between 61 children with sickle-cell disease (SCD) vs. 61 controls, and between 78 human immunodeficiency virus (HIV) –positive children vs. 78 controls.

Results: In virus screening of 102 NPS specimens, 37 (36%) were positive: 27 (26%) for HRV alone, 3 (3%) for HEV alone, and 7 (7%) for HRV+HEV. HRV prevalence was highest during the coolest month, July, 47% (26/53), compared to 22% (8/49) (p=0.021) in April-to-June. In the CSOM study, HIV positivity occurred in 14/22 (64%) of the CSOM children vs. none of the controls (p<0.001) and tuberculosis in 8/22 (36%) vs. none (p=0.002). The most frequent CSOM pathogens were *Proteus* (8/18, 44%) and *Pseudomonas* species (4/18, 22%). CSOM resulted in hearing loss of >25 dB HL in pure-tone averages or BAEP in 17/33 (52%) of the affected ears. In the SCD study of 61 SCD children vs. 61 controls, bilateral hearing loss of >25 dB HL at any frequency occurred in 9/25 (36%) SCD children vs. 3/28 (11%) controls (p=0.047), whereas the prevalence of other ORL findings showed no significant difference. Of 78 HIV-positive children vs. 78 controls, ORL pathology was present in 72 (92%) HIV-positive vs. 61 (72%) control children (p=0.022). Dental caries occurred in 44 (56%) of the HIV-positive children vs. 25 controls (32%) (p<0.001), cervical lymphadenopathy >1 cm in 35 (45%) vs. 8 (10%) (p<0.001), facial skin lesions in 25 (32%) vs. 4 (5%) (p<0.001), CSOM in 21 (26%) vs. 3 (4%) (p<0.001), and bilateral hearing loss of >25 dB HL in PTA or BAEP in 10 (13%) vs. one (1%) (p=0.009).

Conclusions: In children of tropical Luanda, HRV and HEV emerged during respiratory symptoms. CSOM was associated with co-morbidity and hearing loss. Hearing loss occurred in SCD. ORL manifestations of HIV infection were common and various. In resource-poor settings, ORL and hearing services deserve a higher priority.

YHTEENVETO

Taustaa: Korva-, nenä- ja kurkkutaudit (KNK) sekä kuulovauriot ovat kehitysmaiden lapsilla yleisiä mutta puutteellisesti tutkittuja. Siksi näiden sairauksien tausta ja kliininen kuva tarvitsevat tarkempaa määrittelyä.

Potilaat ja menetelmät: Tutkimukseen osallistui vapaaehtoisia eri erikoisalojen poliklinikkapotilaita Angolan pääkaupungin Luandan lastensairaalasta (Hospital Pediátrico). Osallistujilta kartoitettiin lääketieteelliset taustatiedot, tarkastettiin yleis- ja KNK-status sekä tutkittiin kuuloa aivorungon herätevasteiden ja vähintään 5-vuotiailta ilmajohteisen äänesaudiometrian avulla. Sadaltakahdelta hengitystieoireiselta lapselta otettiin nenänielusta tikkunäyte, josta tutkittiin polymeraasiketjureaktiolla rino- ja enteroviruksia. Kahdeksaltatoista kroonista märkäistä välikorvatulehdusta (chronic suppurative otitis media, CSOM) sairastavalta lapselta otettiin bakteeriviljelynäyte. Tutkimustietoa kerättiin ja vertailtiin 23 CSOM:ta sairastavalta lapselta ja 23 ikä- ja sukupuolijakaumaltaan samankaltaiselta verrokilta; 61 sirppisoluanemiaa sairastavalta ja 61 verrokilta, sekä 78 HIV-infektiota sairastavalta ja 78 verrokilta.

Tulokset: Virusnäytteistä 37/102:ssa (36 %) todettiin virus: 27:ssä (26 %) pelkkä rinovirus, kolmessa (3 %) pelkkä enterovirus ja seitsemässä (7 %) molemmat virukset. Rinoviruksen esiintyvyys oli korkeimmillaan viileän heinäkuun aikana 47 % (26/53) ja muina kuukausina (huhti-kesäkuu yhdistettynä) 22 % (8/49) (p=0.021). CSOM-tutkimuksessa HIV-infektio todettiin 14/22 (64%) CSOM-potilaalla eikä yhdelläkään kontrollilapsella (p<0.001), ja tuberkuloosi 8/22:lla (36 %) CSOM-lapsella eikä yhdelläkään kontrollilla (p=0.002). CSOM oli tyypillisesti *Proteus*- (8/18, 44 %) tai *Pseudomonas* -lajien (4/18, 22 %) aiheuttama, ja puhetaajuuksien 0.5-4 kHz kuulovaurio (>25 dB HL) ilmeni 52 %:ssa vuotavista korvista. Sirppisoluanemiasta kärsivillä lapsilla molemminpuolinen, yleensä lievä kuulovaurio oli 9/25:llä (36 %) ja kontrollilapsista 3/28:lla (11 %) (p=0.047); muuten ryhmien välillä ei KNK-löydösten esiintyvyyksissä ollut merkitseviä eroja. Poikkeavia KNK-löydöksiä oli 72/78:lla (92 %) HIV-positiivisella vs. 61/78:lla (72 %) kontrollilapsella (p=0.022); kaulan alueen imusolmukesuurentumia 35 (45 %) HIV-positiivisella vs. kahdeksalla (10 %) kontrollilapsella (p<0.001), iholöydöksiä 25:llä (32 %) vs. neljällä (5 %) (p<0.001), CSOM 21:llä (26 %) vs. kolmella (4 %) (p<0.001) ja molemminpuolista kuulonalenemaa (>25 dB HL) 10:llä (13 %) vs. yhdellä (1 %) (p=0.009).

Johtopäätelmät: Trooppisen Luandan lapsilla tavattiin rino- ja enteroviruksia hengitystieoireiden yhteydessä. Pitkittynyt märkävuoto välikorvasta liittyi usein

yleissairauksiin ja kuulonalenemaan. Sirppisoluanemiaa sairastavilla lapsilla esiintyi kuulo-ongelmia. HIV-positiivisista lapsista valtaosalla oli useita KNK-poikkeavuuksia. Kehitysmaiden lasten KNK- ja kuulo-ongelmiin olisi kiinnitettävä enemmän huomiota.

2. ORIGINAL PUBLICATIONS

This thesis is based on the following articles, which are referred to in the text by Roman numerals [I-IV].

I. Taipale A, Pelkonen T, Roivainen M, Kaijalainen S, Bernardino L, Peltola H, Pitkäranta A. Human rhino- and enteroviruses in children with respiratory symptoms in Luanda, Angola. (Accepted by Paediatrics and International Child Health.)
II. Taipale A, Pelkonen T, Taipale M, Bernardino L, Peltola H, Pitkäranta A. Chronic suppurative otitis media in children of Luanda, Angola. Acta Paediatr 2011; 100: 84-88.
III. Taipale A, Pelkonen T, Bernardino L, Peltola H, Pitkäranta A. Hearing loss in Angolan children with sickle-cell disease. Pediatr Int 2012; 54: 854-857.
IV. Taipale A, Pelkonen T, Taipale M, Roine I, Bernardino L, Peltola H, Pitkäranta A. Otorhinolaryngological findings and hearing in HIV-positive and HIV-negative children in a developing country. Eur Arch Otorhinolaryngol 2011; 268: 1527-1532.

3. ABBREVIATIONS

AOM	Acute otitis media
BAEP	Brainstem auditory-evoked potentials
CSOM	Chronic suppurative otitis media
HEV	Human enterovirus
HIV	Human immunodeficiency virus
HRV	Human rhinovirus
MEE	Middle-ear effusion
NPS	Nasopharyngeal smear
ORL	Otorhinolaryngological
PTA	Pure-tone average
PCR	Polymerase chain reaction
RTI	Respiratory tract infection
SCD	Sickle-cell disease
TM	Tympanic membrane
WHO	World Health Organization

4. INTRODUCTION

In developing countries, otorhinolaryngological (ORL) diseases and hearing loss are highly common. Despite their frequency, they remain, however, widely neglected. Some developing countries have no ORL specialists at all, and even the best served areas cannot be compared to the industrialized world. The shortage of ORL services includes not only medical specialists, but also the ORL knowledge of local general practitioners and nurses, as well as the facilities and technical equipment required. The situation is particularly poor in the audiology field. The brain-drain phenomenon, challenging the health care of many underserved regions, also extends to otolaryngology.[1,2]

This imbalance sets the young populations of resource-poor countries in an especially disadvantaged position, as many ORL diseases typically occur in childhood. Furthermore, the course of these diseases is often prolonged and complicated in children affected by poor hygiene, deficient nutrition, and chronic systemic illnesses. In 1996, the World Health Organization (WHO) held an expert meeting to develop strategies for prevention of deafness due to chronic otitis media in developing countries,[3] followed by a workshop concerning hearing aids a couple of years later.[4] Unfortunately, the medical and political awareness of the impact of untreated ORL diseases on the resource-poor societies has remained low.

Today, medical research concerning developing countries is still largely concentrated on life-threatening conditions such as severe infections. Meanwhile, data on basic epidemiology and the clinical picture of many common ORL diseases are sparse, noncomprehensive, or lacking. However, advances in health care have provided a better prognosis for huge numbers of underprivileged children, making research and management of their less fulminant diseases even more relevant.

The present study was launched in Luanda, the capital of Angola, to achieve epidemiological and clinical data on childhood ORL diseases, specifically related to picornavirus infections, CSOM, sickle-cell disease (SCD), and human immunodeficiency virus (HIV). The republic of Angola is located in southwestern Africa (Figure 1) and is currently experiencing a reconstruction era after its devastating 27-year civil war ending in 2002. Formerly part of the Portuguese Overseas Empire and a major sufferer from the Atlantic slave trade, the country became independent in 1975. Its steady economic growth, mainly fuelled by its rich diamond and petroleum resources, has, however, failed to benefit the vast majority of the population, such as the millions of inhabitants of the shantytowns of Luanda set up by inland escapees from the civil war (Table 1).[5]

In the present study, it very soon became evident that medical research in resource-poor settings has as typical challenges how to import the required equipment

Figure 1. Climate map of Africa, showing location of Luanda (figure by author).

Table 1. Comparison of country profiles of the Republic of Angola and Finland.[5]

	Angola	Finland
Population	19.1 million	5.3 million
Urbanized proportion of population	59%	85%
Life expectancy at birth	52 years	79 years
Under-five mortality rate	161/1000	3/1000
Number of HIV-positive children (estimated)	22 000	-
Conceptions affected by SCD (estimated)	16/1000	-
Gross national income per capita	3 960 US$	47 170 US$
General government expenditure on health	5.3%	11.7%
Proportion living under the international poverty line (1.25 US$/day)	54%	Unknown
Adult literacy rate	70%	100%

through customs, how to recruit and communicate with people who are commonly unfamiliar both with the concept and purpose of research and with basic medical knowledge, how to manage problems of electricity supply and electronic devices without technical expertise available, and how to make short-term research locally useful and ethically sustainable. These challenges may in part explain the persistence of medical inequity between developed and developing world, but it should by no means prevent the worldwide efforts to reduce it – may the present study thus join in the mission with its own little share.

5. REVIEW OF THE LITERATURE

5.1 Otorhinolaryngological services, audiology, and research in resource-poor context

Even though the developing world stretches over virtually all the populated continents, the similar limitations in living conditions and health care systems in this vast area make the emerging ORL problems surprisingly similar. Therefore, in spite of the huge geographical, ethnic and cultural differences, it is no mere generalization to use as a common denominator "developing countries" for these regions. Typically, developing countries foster young, rapidly growing populations with broad ethnic and cultural diversity which suffer from high income inequalities. Domestic infrastructure, education, and manufacturing capacity seldom meet the demands of health care for these populations.[6] In practice, the restricted resources are often directed toward the most severe and lethal conditions, whereas diseases leading to disability but only rarely to immediate mortality are left with little attention.[7]

The developing world is therefore characterized by a marked shortage of ORL services, personnel, and training opportunities. For example, in a survey covering 18 African countries (not including Angola) with known public ORL services, the number of people per one ORL specialist ranged from 240 000 (South Africa) to 10 million (Malawi), in comparison to the 1:100 000 in the UK, which, in fact, ranks at the bottom among Western countries.[8]

Of these 18 African countries, 10 offered national ORL training, 2 had a training program for audiologists, and 12 had no or very poor ORL services outside major cities. The availability of various services was zero or poor as follows: audiology in 14 of the 18 participating countries, brainstem auditory-evoked potentials (BAEP) measurement in 16, hearing screening at schools in 15, hearing aids in 16, bone-anchored hearing aids in 17, cochlear implants in all 18, myringotomies/ventilation tubes in 14, tympanoplasty in 12, and mastoidectomy in 12. The authors concluded that in order to reach the lowest European level for ORL services, nearly 4800 additional ORL specialists are required in sub-Saharan Africa, and proposed establishing a worldwide "Developing World Forum for ENT Surgery, Audiology, and Speech Therapy" to reinforce the clinical, teaching, and research resources in ORL in developing countries.[1,2]

Annually, nearly 800 000 babies (6/1000 live births) are born in developing countries are now suffering or eventually will suffer from prelingual hearing loss.[7,9] By the age of nine, hearing loss prevalence will probably at least double.[7] Roughly

half these cases are believed to be preventable, and otitis media emerges as a major avoidable etiology alongside meningitis, cerebral malaria, measles, and mumps.[7,9,10] Usually, public awareness of the signs, consequences, and rehabilitation of childhood hearing loss remains low, and, in the absence of widespread hearing screening programs, the handicap may lead to permanent limitations in their linguistic and educational development, economically burdening already poor societies.[3,7,10] Outside the Western countries, only China, India, and Botswana produce hearing aids locally; generally, retail prices of imported devices remain unaffordable for customers in developing countries.[11]

Nationwide, systematic hearing screening programs are lacking in developing countries. Regional neonatal hearing screening programs have recently been launched in some resource-restricted areas,[12] but generally hearing-screening in developing countries relies on conventional audiometry typically performed in schools.[9] Simple low-cost, hand-held audiometers are available in developing-country settings.[9,10] In addition, screening takes place in immunization clinics.[13]

To save resources, screening has been targeted at known risk groups.[12,14]

Children at risk for hearing problems have also been identified by use of specific questionnaires on known risk factors and the guardian's observations of the child's speech development and response to sounds.[15,16] Increasingly, physiological measurements such as BAEP and otoacoustic emissions have also become available in resource-restricted countries.[17]

In general, relatively few ORL studies have been conducted in developing countries. In the absence of local databases of childhood ORL diseases, large population-based epidemiological surveys appear rarely and are available only from certain regions. Such reports typically concerned hearing loss and ear pathology in schoolchildren. No cohort-based studies exist. Epidemiological data on ORL diseases in younger children also prove difficult to find. Since long-term follow-up was typically impossible, prevalence rather than incidence rates were generally reported for ear and hearing pathology. Another type of report available was hospital-based, comprising retrospective chart-reviews and prospective studies, mainly conducted in tertiary referral centres of major cities where local ORL services exist. These studies therefore probably concerned the more severe and complicated cases, or cases occurring in generally ill children, because in developing countries medical aid for ORL diseases is often sought only as a last resort.

5.2 Global burden of human rhino- and enteroviruses in respiratory infections

Human rhino- (HRV) and enteroviruses (HEV) are major pathogens in paediatric respiratory tract infections (RTI) in temperate climates of the northern hemisphere.[18-23] Being members of the *Enterovirus* genus and *Picornaviridae* family and thus

being only 30 nanometers in diameter, HRV and HEV carry single-stranded RNA inside an icosahedral, non-enveloped capsid consisting of four glycoproteins (VP1-4).[24-26] HRV is further classified into 3 (HRV-A, HRV-B, and HRV-C) and HEV into 4 (HEV-A-D) species, comprising more than 100 HRV and HEV (sero)types.[25,27]

The vast majority of HRV and several HEV types enter human cells through the intercellular adhesive molecule (ICAM-1) receptor[25,28] in the oral mucosa or upper respiratory epithelium. HRV is transmitted through contact with contaminated hands or with inanimate surfaces,[20,22,29] where it may persist for hours, even days.[29,30] HRV and HEV may be identified by cell culture or reverse transcription polymerase chain reaction (PCR), the latter being superior in sensitivity.[20,31]

The clinical profile of HRV infection involves the typical common cold symptoms of rhinorrhea, sneezing, and cough, and appear either alone or in combination with bacteria, and also rhinosinusitis, otitis media, and pneumonia.[22,24,27,32-34] In addition, HRV associates with asthma exacerbation in children.[35,36] HRV, being acid-sensitive, is unable to survive in the low gastric pH.[20,22,27] Some recent data suggest that HRV-C may enter the circulation,[37] but typical HRV infections are restricted to respiratory tissues. HEV also typically first invades the respiratory tissues, but, apart from common cold symptoms, it may equally likely cause gastroenteritis, "foot-and-mouth disease", and severe, even fatal infections such as myocarditis and meningoencephalitis.[24,38,39]

In a child living in a temperate climate, picornaviruses cause an estimated five symptomatic infections per year,[40] and account for approximately half of the yearly common cold episodes.[31,40] Their incidence is, however, seasonal, with studies reporting proportions of 25 to 71% for HRV,[18,19,23,32] and 10 to 18%[18,23] for HEV in common colds. In temperate regions, HRV occurs throughout the year, but its incidence tends to peak in autumn,[40-44] and in some regions, also in spring.[42-44] Factors contributing to increased HRV transmission include high relative humidity[45,46] and crowding of children indoors during school terms.[22,43]

In general, little data exist on the seasonality of HEV-caused common colds. HEV serotypes responsible for more severe diseases may either emerge in an epidemical pattern, prevail in summer-autumn, or express no clear seasonality,[24,38] and generally no seasonality have been recorded for common colds from HEV.[41,47]

Data on common colds in tropical and subtropical climates are limited. However, the reported prevalences for HRV and HEV among children with respiratory symptoms range from 22 to 52% [48-51] and 3 to 15%[49,50,52] among outpatients/follow-up cohorts (Table 2), and 6.5 to 41%[48,53-55] and 4 to 20%[53,54] among hospitalized children. In the tropics, HRV occurrence reportedly increases with humidity or rainfall[53,54] and cooler temperatures,[53] and HEV with dryness and hot temperature,[54] but several studies found no clear seasonality pattern for HRV[48,49,55] or HEV.[49,50,53,56]

Picornaviruses are highly associated with otitis media in children: HRV has occurred in nasopharyngeal or middle ear samples of children with acute otitis media (AOM) in 20 to 41%[18,41,57,58] and HEV in 6 to 25%.[41,58,59] On the one hand, a follow-up

Table 2. Human rhinovirus (HEV) and human enterovirus (HEV) in paediatric outpatients of subtropical/tropical low-resource countries in the polymerase chain reaction era.

Country, year	N	Age (years)	Sampling method	HRV (%)	HEV (%)
Mozambique 2011 [49]	333	0–1	NPA	26	3
Kenya 2012 [48]	254	0–12	NPA	22	unmeasured
Kenya 2010 [51]	197	5–10	NPS	26.4	unmeasured
Senegal 2010 [220]	82	0–5	NPS	9.7	0

NPA, nasopharyngeal aspirate
NPS, nasopharyngeal swab

study of children under age two detected picornaviruses in MEE or nasopharyngeal samples of 54% of all children with AOM,[41] while on the other hand, another study showed that 30 to 34% of picornavirus colds were accompanied by AOM.[18] No data emerged on otitis media in picornavirus colds in children in tropical climates.

5.3 Chronic suppurative otitis media in children of developing countries

5.3.1 ETIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF CHRONIC OTITIS MEDIA

Chronic suppurative otitis media (CSOM) is a persistent inflammation of the mucosa and submucosa of the middle-ear cleft which produces a suppurative discharge through a chronically perforated tympanic membrane (TM).[10,60-63] The WHO defines otorrhoea as chronic after it endures for at least 2 weeks,[10] but no concerted opinion exists on the cut-off duration, and various values up to 3 months have been suggested.[3,61,64] The bacterial etiology of CSOM, reflecting either primary infection or secondary colonization, involves *Staph.aureus* and *P.aeruginosa,*[10,60,65-69] but children from a low-hygiene environment also exhibit enteric (e.g. *E.coli*) and low-virulence (e.g. *Proteus*) Gram-negative rods.[60,70-73] In addition, anaerobes may contribute in up to 71% of cases.

In the antibiotic era, CSOM has become a disease of the developing world. In addition, certain subpopulations in economically developed areas, such as the Inuits, South Pacific islanders, and Australian aboriginals, exhibit particularly high CSOM prevalences: up to at least 15%.[74-78] The vast majority, approximately 85 to 90%, of the global CSOM burden lies on Asia and South Pacific, mainly due to the large population of these areas, whereas the continent of Africa constitutes 4 to 8% of the cases worldwide. The WHO classifies CSOM prevalences as the following: the lowest <1%, low 1 to 2%, high 2 to 4%, and highest >4%; with prevalences exceeding 2% demanding urgent attention and >4% representing “a massive public health problem”.[10] Sub-Saharan Africa has reported prevalences of 1.1 to 4.6%,[10,79-82]

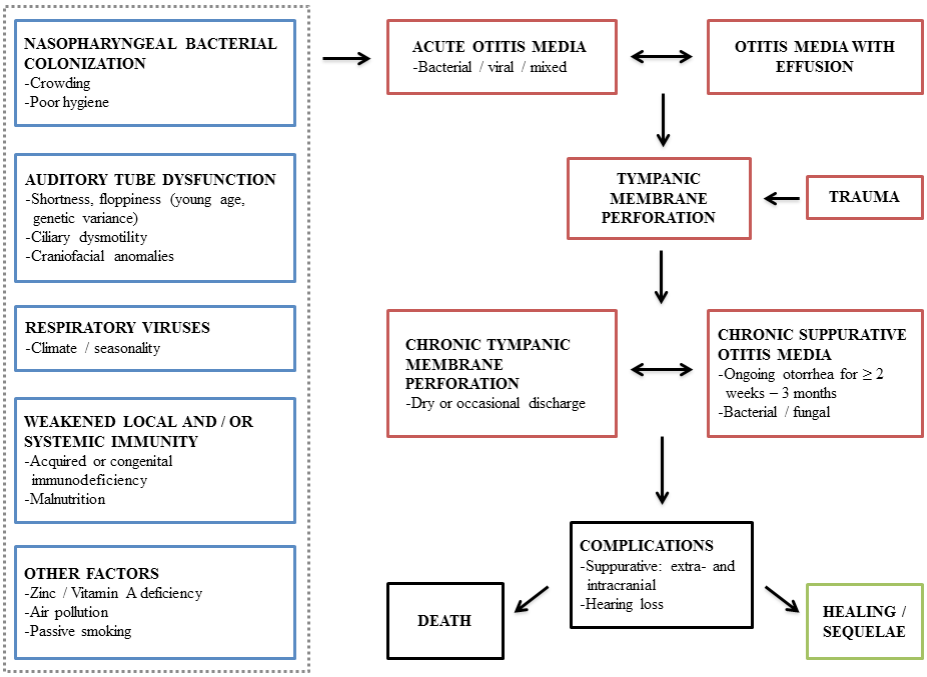


Figure 2. Risk factors, features, and events contributing to progression of otitis media towards chronic and complicated forms in children of developing countries.

contrasted with the European WHO estimate of 0.4%.[10] In Angola, in the 1980's, chronic otitis media occurred in 3.4% of schoolchildren.[79] Despite its relatively low global share in CSOM cases, of all deaths due to otitis media complications in 2008, Africa accounted for two-thirds.[83]

Approximately one-fifth of the CSOM cases occur in children less than age 5.[84] Risk factors for chronic otorrhea include poor socio-economic status, crowding, malnutrition, short duration of breastfeeding, parental smoking, and lack of clean water for washing.[72,75,77,78,82,85] In resource-poor settings, deficiencies in vitamin A and zinc may also add to the risk for a chronic or more severe course of otitis media.[86-88] In addition to the living conditions and environmental variables, genetic factors predispose to chronic otitis through anatomical (e.g. short auditory tube)[60,63] and functional (e.g. epithelial ciliary motility) variance[60] (Figure 2).

In general, few data exist on treatment of paediatric CSOM in developing countries. The strongest evidence concerns the resolution of otorrhea, while in the lack of long-term follow-up, data on recurrence, restoration of the tympanic membrane and hearing, complications, and adverse effects of the treatment remain uncertain.[89] However, the safety of topical ofloxacin is evident in children.[90]

In resource-poor settings, the treatment is based on regular ear cleansing, which in itself has no therapeutic efficacy,[91] but facilitates the entry of topical antimicrobials into the middle ear.[92] Clinical trials have shown the efficacy of ototopical an-

tibiotics in treating CSOM in children;[89] at least ofloxacin,[70,91,93] ciprofloxacin,[68,94] and neomycin-polymyxin-B[93] have been studied in low-resource settings. Other topical antibiotics in clinical use include gentamycin, tobramycin, chloramphenicol, trimethoprim, and erythromycin.[10] Adding systemic antibiotics to local treatment is not recommended due to its lack of efficacy and low cost-effectiveness.[10] Topical antiseptics, such as boric acid, are cheap and useful,[95] but remain less efficacious and may cause more local irritation than a local antibiotic would.[94]

5.3.2 COURSE AND CONSEQUENCES OF CHRONIC OTITIS MEDIA

Generally, CSOM is regarded as forming a continuum with AOM and otitis media with effusion,[60,61,63] and in developing countries it is mostly believed to arise from an unresolved or untreated AOM episode, whereas traumatic TM perforations may play a lesser role.[3,10,63,96] In industrialized countries, CSOM usually associates with tympanostomy tubes. [97,69,98,99] In otitis-prone children, early bacterial colonization, leading to formation of biofilms and chronic inflammation in the nasopharynx and auditory tube, may contribute to the persistence of middle-ear infections.[100,101]

Prolonged inflammation of the middle-ear cavity induces changes in the surrounding structures that complicate treatment attempts and the healing process. In the mucosal lining, metaplasia occurs, and mucus-producing glandules, as well as ciliated cells and highly vascular granulation tissue emerge. Ongoing infection may also invade the underlying bone, causing osteitis and reactive osteogenesis. Pathological changes detectable in TM include fibrosis, thickening, retraction, epithelialization, and myringitis that may lead to polyp formation. Cholesteatoma may also occur.[10,61,62,102,103]

CSOM may lead to conductive hearing loss resulting from the discharge in the ear canal. Thickening, retraction, and perforation of the eardrum also produce conductive hearing loss.[3,61] In addition, resorption and disruption of the ossicular chain, in 11 to 68% of patients of various ages discovered during surgical treatment,[104-106] may worsen hearing loss. Bacterial toxins may also enter the inner ear through the round window membrane, causing cochlear damage and a sensorineural or mixed type of hearing loss.[107-109] Another risk factor for cochlear hearing loss is ototoxicity from topical aminoglycosides, which are usually preferred to the safer but more expensive quinolones in lower-resource health care.[3] However, ototoxicity may not be very usual in clinical settings.[110]

Apart from hearing loss, CSOM may lead to suppurative complications that can be extracranial, such as labyrinthitis, mastoiditis/mastoid abscess, petrositis or facial paralysis; or intracranial, such as thrombosis of lateral or cavernous sinus, meningitis, cerebellitis, epidural/subdural/cerebral abscess, hydrocephalus, and labyrinth sclerosis. These intracranial complications hold the main responsibility

for CSOM-associated mortality, which in Africa has been estimated by the WHO to reach 1.8%.[10,84,102,103,111,112]

In sub-Saharan Africa, up to 3100 children under 15 thus far have died annually in the 2000's due to complications of otitis media.[84] Even though severe complications of CSOM have been estimated to be relatively rare in children,[10] their risk is significantly higher than for adults[84,111] Among CSOM patients presenting at ORL clinics in Africa, subperiosteal abscesses and intracranial complications have been reported in 6 to 11%.[103,111,112] Apart from mortality, complications of CSOM may lead to severe permanent neurological sequelae.[10]

In Thailand, 102 (0.59%) complications occurred in 17 144 otitis media cases seen in health care settings in the 1980's, and the majority of them arose from CSOM. In that series, the most frequent extracranial manifestations were facial paralysis, subperiosteal abscess, and labyrinthitis, whereas the intracranial complications were mainly meningitis and intracranial abscess, leading to death in 18.6%.[113]

However, chronic suppuration may also spontaneously resolve, followed by healing of the perforation or by chronic perforation with either no or only an occasional discharge.[63] In follow-up of CSOM children without therapy, suppuration had ceased at 6 weeks in 18%[72] and at 16 weeks in 22%[91]; and only 13% had intact TMs at 16 weeks.[91]

Of the worldwide otitis media-related hearing loss, 90% occurs in developing countries, where CSOM appears a major cause for acquired hearing loss in children.[3,80,96,114] Among paediatric CSOM patients, hearing loss has been documented in 47 to 96%,[80-82,114-116] and mainly presents as slight or moderate.[68,81,82,114] CSOM affects most heavily young children,[3,72,85,112,115] and may endanger their language acquisition, social development, and scholastic performance.[75,82,117]

5.4 Otorhinolaryngological signs and complications of sickle-cell disease

5.4.1 EPIDEMIOLOGY, PATHOPHYSIOLOGY, AND MANAGEMENT OF SICKLE-CELL DISEASE

Sickle-cell disease (SCD) is the most prevalent monogenic disease worldwide, with 300 000 affected children being born every year.[118] Of the patients, the developing countries have at least 80%.[119] In Sub-Saharan Africa, 180 000 births occur annually, and due to improved patient survival, the burden of disease in this area is increasing.[119] Given survival rates half the local average, six million SCD patients will live in the area.[120] The generally healthy carriers of the sickle-cell mutation, "trait", appear to be nearly 90% protected against severe *Plasmodium falciparum* malaria,[121] and the malaria-endemic regions of Arabia, Mediterranean, India, and Africa thus experience the highest SCD incidence.[120,122-124]

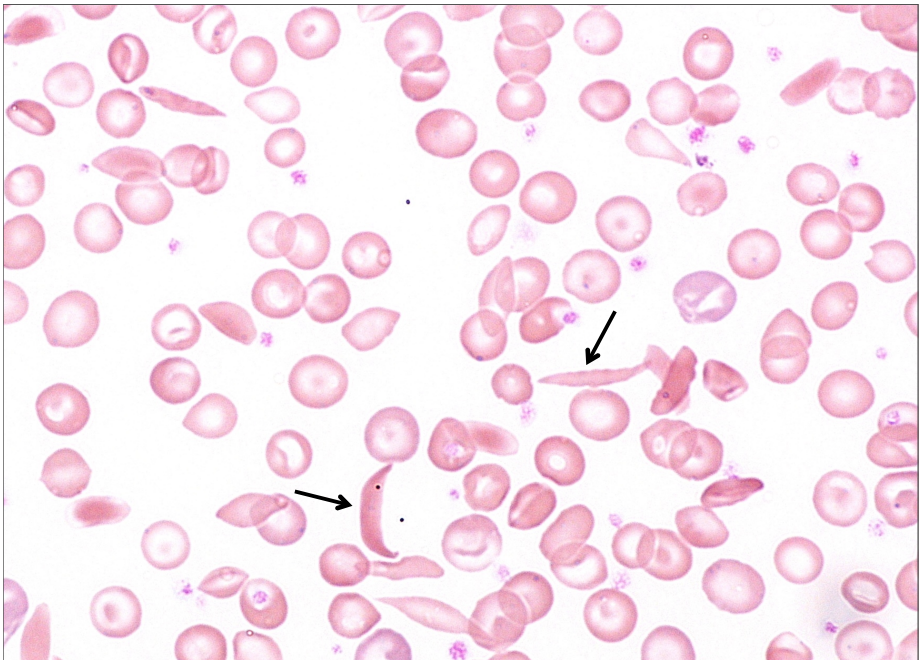


Figure 3. Abnormally shaped erythrocytes in sickle-cell disease (figure provided by specialist in clinical chemistry Päivi Helminen-Pacius from HUSLAB laboratory, Helsinki University Central Hospital, Finland).

In Africa, the top SCD rates occur between 15° north and 20° south latitudes where up to 40% of the population may carry the sickle-cell trait.[125] In Angola, SCD affects 1.6% of conceptions,[123] which may exceed the actual proportion of newborns, because SCD adversely affects fetal survival.[126]

SCD is a haemoglobin disorder resulting from coinheritance of the "sickle" allele, HbS, and another mutant haemoglobin allele; either a second HbS resulting in the homozygous HbSS disease called sickle-cell anemia, or another type of a mutant allele, such as HbC or thalassemia, resulting in compound heterozygous inheritance.[120,124,127] In sub-Saharan Africa, the HbSS genotype prevails, with only small minorities of HbSC and other types.[122,125]

The sickle haemoglobin contains a single amino acid substitution (valine for glutamic acid) in its β-chains, leading to loss of form and function of the red blood cells (Figure 3), chronic haemolytic anemia, and acute vaso-occlusive crises, as well as various systemic outcomes related to haemolysis and tissue infarctation.[124,127] Through vaso-occlusive congestion, repeated splenic infarcts, and fibrosis, SCD leads to functional hyposplenism,[128] which makes the children highly susceptible to severe bacterial infections, particularly by *Streptococcus pneumoniae*,[123,129-132] which markedly contributes to the estimated 50 to 90% mortality among affected African children.[122,133,134] In addition to the genetic factors, environment plays a marked role in the phenotype of SCD patients,[135] and a paucity of data exists on the complications and natural course of SCD in developing countries.[119]

Table 3. Hearing loss exceeding 25 dB HL in audiometry, and related middle-ear pathology (MEP) in children with sickle-cell disease. Adapted from: Taipale A, Pelkonen T, Bernardino L, Peltola H, Pitkäranta A. Hearing loss in children with sickle-cell disease. *Pediatrics International* 2012; 854-857. © The Authors. Pediatrics International © 2012 Japan Pediatric Society.

Author, country	N	Age (years)	Hearing loss		Proportion of MEP in hearing loss
			n (%)	bilateral	
Alabi et al., 2008 (139) Nigeria	80	4–15	25 (31%)	22	28%[a]
Odetoyinbo and Adekile, 1987 (144) Nigeria	56	6–15	12 (21%)	7	0%
Mgbor and Emodi, 2004 (143) Nigeria	52	6–19	7 (13%)	7	no mention
De Castro Silva et al., 2010 (140) Brazil	40	8–20	11 (28%)[a]	5	0%
Tsibulevskaya et al., 1996 (138) Kenya	33	7–14	12 (36%)	2	0%
MacDonald et al., 1999 (149) USA	84	8 months – 24 yrs	26%[c]	NM	23%[a]
Forman-Franco et al., 1982 (141) USA	54	12 (mean)	11%	NM	7%

[a] Middle-ear effusion
[b] Hearing loss exceeding 20 dB, measured by brainstem auditory-evoked potentials
[c] Neither number of children with nor definition of hearing loss mentioned

Successful management of SCD is possible in resource-poor settings and includes a balanced diet with folic acid supplement, avoidance of extreme physical stress, heat, and dehydration, treatment with penicillin prophylaxis, pneumococcal vaccinations, analgetics, hydroxyurea, and blood transfusions.[118,125,136] Newborn screening, not yet usual in developing countries, is necessary for early identification and management of new SCD children,[137] and has the potential to reduce SCD mortality.[138]

5.4.2 HEARING LOSS IN SICKLE-CELL DISEASE

SCD is known to associate with sensorineural hearing loss in children,[139-144] adolescents,[140,141,143-149] and adults.[146-152] Hearing loss has been proposed to relate to cochlear ischaemia, based on post-mortem observations of the temporal bones of an SCD child with known hearing loss.[153] Corroborating this theory are case reports of adolescents with reversible hearing loss in association with vaso-occlusive crisis.[141,154] Other support for hypoxia in the pathogenesis of SCD-related hearing loss include the high dependency of cochlear homeostasis on its vascular supply, inner-ear vascularization deriving exclusively from the internal auditory artery, and the degeneration and ossification in the cochlea after internal auditory artery occlusion.[155] In one SCD child, labyrinthitis ossificans has been a cause of progressive sensorineural hearing loss.[156]

Hearing loss of varying definitions has occurred in SCD children/adolescents at a rate of 3.5 to 12% in developed countries.[142,157-159] In developing countries, conductive or sensorineural bilateral hearing loss exceeding 25 dB HL has been reported in 6 to 27.3% of mainly school-aged children (Table 3). Some studies have perceived higher prevalences of hearing loss in older patients.[147,148,150,152,160] The SCD-related hearing loss has been reported to involve either exclusively high,[145,147-149,160] or all[142-144,146,154,160,161] frequencies, with both sudden[141,154] and slow[145,147] onset. The sudden-onset hearing loss may relate to an acute vaso-occlusive event in the cochlea,[141,154] whereas the slow-onset type may result from anoxia of the hair cells produced by non-acute erythrocyte sickling and chronically impaired local oxygen balance.[162]

5.4.3 OTHER OTORHINOLARYNGOLOGICAL MANIFESTATIONS IN SICKLE-CELL DISEASE

The ORL manifestations of SCD remain poorly described in children. The estimation is that unvaccinated SCD children without prophylactic penicillin may experience a 33- to 100-fold[123,132,163] risk for invasive pneumococcal infections. *Streptococcus pneumoniae* frequently causes otitis media, which is, in contrast, usually a localized infection, and no studies have been aimed at defining the incidence or prevalence of otitis media in SCD children. However, antibody-deficient children without haemoglobin disorders do suffer from recurrent and persistent ear infections.[164,165]

SCD-related ORL findings, mainly described in case reports, include bone infarcts, osteonecrosis, osteomyelitis, extramedullary hematopoiesis in the paranasal sinuses, bone marrow hyperplasia of the maxilla and skull base, soft-tissue abscesses, and cervical lymphadenopathy.[166-169] Adenoid hypertrophy[170,171] and nasal obstruction resulting from turbinate congestion[170] may also occur in children. In the absence of prospective follow-up studies, the frequencies of various manifestations remain, however, unknown.

5.5 Otorhinolaryngological pathology in human immunodeficiency virus –infected children

5.5.1 PATHOPHYSIOLOGY, EPIDEMIOLOGY, AND MANAGEMENT OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Human immunodeficiency virus (HIV) is an enveloped, single-stranded RNA retrovirus in the *Lentivirus* genus of the Retroviridae family. With the aid of its external gp120 glycoprotein, HIV enters CD4 (cluster of differentiation 4) glycoprotein-expressing human cells, e.g. helper T cells, monocytes, macrophages, and glia cells. Initial infection occurs through percutaneous or mucous membrane contact with contaminated objects or body fluids, as well as transplacentally in utero. Untreated

Table 4. World Health Organization clinical stages of human immunodeficiency virus (HIV) infection.[177]

Clinical stage	General findings	Otorhinolaryngological findings
I – Asymptomatic	• Persistent generalized lymphadenopathy	• Persistent cervical lymphadenopathy
II – Mild	• Herpes zoster • Papular or seborrhoeic dermatitis • Extensive wart virus or molluscum contagiosum infection • Persistent hepatosplenomegaly	• Recurrent sinusitis, tonsillitis, otitis media, pharyngitis • Angular cheilitis • Lineal gingival erythema • Recurrent oral ulceration • Persistent parotid enlargement
III – Advanced	• Moderate malnutrition or wasting • Persistent fever or diarrhoea • Pulmonary or lymphatic tuberculosis • Recurrent bacterial pneumonia • Chronic HIV-associated lung disease • Anaemia, neutropaenia, or chronic thrombocytopaenia	• Acute necrotizing stomatitis or gingivitis • Persistent oral candidiasis • Oral hairy leukoplakia • Cervical lymph node tuberculosis
IV – Severe	• Severe malnutrition, stunting or wasting • Chronic cryptosporidiosis, isosporiasis, or herpes simplex infection • Recurrent severe bacterial infection • *Pneumocystis jirovecii* pneumonia • Atypical mycobacteriosis, extrapulmonary tuberculosis or cryptococcosis, disseminated mycosis • HIV encephalopathy • Kaposi sarcoma, Non-Hodgkin lymphoma	• Chronic herpes simplex infection (orolabial) • Oesophageal candidiasis • Kaposi sarcoma (oropharyngeal, intraparotid) • Non-Hodgkin lymphoma (intraoral, cervical)

infection leads to deterioration of the host's immune system, subsequent severe bacterial and viral infections, diseases by opportunistic microorganisms (candida, *Pneumocystis jirovecii*, *Cryptococcus*, mycobacteria), occurrence of malignancies, general wasting, and, finally, death.[172]

In resource-poor countries where children are usually infected intrauterinally, perinatally, or via breastfeeding, roughly half the untreated children progress to death within the first 2 years of life,[173,174] while the rest appear to be slow progressors with a median survival of 6 years, or long-term survivors with several asymptomatic years.[172] Paediatric HIV is mainly being caused by the viral subtype HIV-1, whereas HIV-2 is of West African origin and occurs rarely in children.[175]

Worldwide, sub-Saharan Africa carries the heaviest burden of HIV. In 2009, 2.3 million HIV-positive children lived in the area, constituting more than 90% of

Table 5. Percentages of otorhinolaryngological findings in representative studies in HIV-positive children. Adapted from: Springer and *European Archives of Oto-Rhino-Laryngology*, volume 268 (2011), p.1527-1532. Otorhinolaryngological findings in Human immunodeficiency virus-positive and –negative children in a developing country. Taipale A, Pelkonen T, Taipale M, Roine I, Bernardino L, Peltola H, Pitkäranta A., Table 4; with kind permission from Springer Science and Business Media.

Author, country	N	Cervical lymphadeno-pathy	Eczema	Chronic OM	Parotid hyper-trophy	Oral candidiasis	Any ORL[b] pathology
Chaloryoo et al. (1998) [186] Thailand	250	42	23	18	5	60	NM[c]
Gondim et al. (2000) [188] Brazil	20[a]	60	NM	15	25	35	90
Hadfield et al. (1996) [185] UK	66	71	15	8	14	33	91
Singh et al. (2003) [184] UK	107	36	NM	NM	6	18	50

OM, otitis media
ORL, otorhinolaryngological
NM, not mentioned
[a] Mean age 3.8 years

all paediatric HIV cases. Both the incidence of and mortality from paediatric HIV declined slightly in the area during the 2000's, resulting in a net increase in the number of children living with HIV. In fact, two-thirds of the world's HIV patients live in the area of 10 sub-Saharan countries including Angola. The number of Angolan HIV-positive children reached 22 000 in 2009, at a time when only some 15% of these children received antiretroviral therapy.[176]

In children at least 18 months old, HIV may be diagnosed serologically, whereas – due to the persistence of maternal antibodies a definite diagnosis in younger children requires virological tests.[177] A positive serological test in an infant, however. reveals exposure to HIV.[178] In low-resource health care, where the availability of virological testing is constrained, clinical signs[178] or other parameters[179] may be necessary to predict the HIV status of the infant. In developed countries, HIV staging is based on viral PCR and CD4 T cell counts, which are however not always available in resource-poor settings. Therefore, based on clinical signs, WHO has staged the severity of HIV infection in four categories (Table 4).[177]

Medical management of HIV infection is based on combination therapy with antiretroviral drugs, called, by their mechanism of action, nucleoside and non-nucleoside reverse-transcriptase inhibitors, and protease inhibitors. In children under age 2, antiretrovirals should be started promptly after diagnosis, whereas in older children, the beginning of treatment should be guided by CD4 counts.[178] Zidovudine prophylaxis in the newborn has been used for prevention of intrapar-

tum HIV transmission, but a two- or three-drug regimen has proven more efficacious if the mother has not received antepartum antiretroviral therapy.[180] However, in low-income settings, limitations in infrastructure, drug availability, monitoring resources, and treatment adherence challenge HIV prevention and management.[175]

5.5.2 OTORHINOLARYNGOLOGICAL ASPECTS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Worldwide, more than 90% of newly infected HIV children live in sub-Saharan Africa,[176] and at least 22 000 HIV-infected children live in Angola.[181] Paediatric HIV manifests in ORL area in multiple ways[182-189] and in up to 91% of the infected;[184,185,188] often already during the first years of life.[188] Table 5 summarizes previously documented frequencies of common ORL findings among HIV-positive children of developing countries, and Table 4 presents the ORL aspects of the HIV staging system. In addition to the manifestations mentioned in these tables, marked adenotonsillar enlargement may occur in HIV children,[182] as well as initial hypertrophy followed by end-stage atrophy.[190]

Hearing loss has been reported in HIV-positive children in 27.5 to 38.8%,[191-194] and sensorineural hearing loss in 7.1 to 16.7%.[192-194] Possible causes for sensorineural hearing loss in HIV patients include ototoxicity from antiretroviral therapy (mainly nucleoside analogues, as reported in adults), antituberculotic medication (streptomysin),[195,196] aminoglycosides,[195,197,198] or possibly the direct neurotoxic effects of the HIV infection itself.[192-194] In addition, cytomegalovirus and *Cryptococcus* infections have induced hearing loss in HIV-positive adults,[199,200] with no reports in children.

Despite the possible presence of cochlear and neural lesions from these causes, however, a major contributor in HIV-positive children's hearing problems is otitis media, which strongly associates with HIV both in developed[187,201-203] and in developing[191,204-206] countries. The severity of immunodeficiency may correlate with a tendency for chronic[204,207] or recurrent[202,203] otitis media. In African HIV-infected children seen at hospital, 4 to 20% have presented with otorrhea,[206,208,209] which usually involves bacteria typical of CSOM in developing countries.[186,210] HIV-infected children are also subject to complications of otitis media.[187]

6. AIMS OF THE STUDY

The primary aim of the present study was to collect epidemiological and clinical data on paediatric ORL findings in Luanda, Angola. The specific aims were to assess:

- the prevalence of and clinical findings concerning human rhino- and enterovirus positivity during the cool season;
- CSOM-related background characteristics, bacteriology, and hearing;
- ORL status and hearing in SCD patients;
- HIV-associated ORL findings and hearing.

7. CHILDREN AND METHODS

7.1 Study groups and laboratory methods

The study data were collected in the Paediatric Hospital of Luanda (Hospital Pediátrico David Bernardino), a university teaching hospital, in January-July 2008. The Paediatric Hospital's ethics committee gave approval for the study on December 27th 2007. The study children were recruited among voluntary outpatients of the Paediatric Hospital's different polyclinics (pulmonology, surgery, general pediatrics, neurology, HIV, sickle-cell disease, gastroenterology, infectious diseases). In addition, a few accompanying siblings of the patients were included. At the general enrollment desk for all clinic outpatients, the receiving secretary provided basic information on the study and offered the opportunity for everybody to participate. Children with ear discharge, as well as outpatients of the HIV and SCD clinics were addressed in particular. In addition, a local nurse who served as a study assistant helped in the recruitment. All volunteering children were primarily accepted, and the children or their guardians then received more specific oral information on the aims and procedures of the study from the examining doctor prior to giving their oral consent.

During the study period, data came from a total of 406 children. All children meeting the inclusion criteria for Studies I to IV were then extracted from this data pool. In Studies II to IV, the age- and gender-matched controls for the patient groups were randomly selected among the remaining data pool. The control groups in Studies III and IV therefore included 29 children in common.

For Study I, 102 children displaying symptoms of the common cold (rhinorrhoea or cough, with or without fever) were recruited during April-July. Of these children, 79 were participants from Studies II to IV: 65 patients from Studies II to IV and 14 controls from Studies III and IV.

In Study I, a nasopharyngeal smear (NPS) was obtained with a sterile cotton swab from each of the 102 participants. The NPS specimens were preserved frozen, transported to Helsinki, Finland, and analyzed for HRV and HEV by real-time PCR assay. The analysis for HRV was performed as described earlier,[211] applying the cut-off threshold of 8% of the strongest positive control. The analysis for HEV was performed according to the method by the US Centers for Disease Control and Prevention, Atlanta, Georgia, USA[212] applying the cut-off threshold of 10% of the strongest positive control. The real-time-PCR analyses were executed at Finland's National Institute of Health and Welfare.

Study II comprised 23 children with CSOM and 23 age- and gender-matched controls without ear complaints or TM perforations. The CSOM group comprised

children with purulent middle-ear discharge for at least 2 months. Bacterial smears from the discharging ears – or in bilateral cases, from the more profusely discharging ears – were taken with a sterile cotton swab and cultured under aerobic conditions according to the norms of the National Committee for Clinical Laboratory Standards.

For Study III, 61 children with SCD from the SCD outpatient clinic were recruited; 61 age- and gender-matched children without clinical suspicion of SCD, known sickle cell trait, or a previous cerebral infection served as controls. SCD diagnosis was based on haemoglobin electrophoresis. The controls did not routinely undergo haemoglobin analysis.

Study IV comprised 78 HIV-positive outpatients from the HIV polyclinic, and an age- and gender-matched group of 78 controls without clinical suspicion of HIV. HIV was diagnosed with a specific antibody-detection test (Hexagon HIV® Human, Wiesbaden, Germany). The HIV negativity of the controls was not serologically verified.

7.2 Interview and physical examination

All study participants, together with their guardians, underwent a structured interview concerning the child's living conditions and family size, medical history and vaccinations, intake of medication, and ear- and hearing complaints. Subsequently, a basic physical examination and a thorough ORL status were performed (Appendix). The children's linguistic development was assessed in a free conversation with the child; especially with the youngest children, assessment was based on information from the guardian.

The basic physical examination comprised an overall assessment of the child's general condition, measurement of weight and height, cardiac and pulmonary auscultation, and palpation of the abdomen. In addition, the inguinal and axillar lymph nodes were palpated and dermal abnormalities registered. The children's nutritional status was assessed by the WHO standards for weight for age, and children with a low weight for age (below -2 SD or the 3rd percentile) were considered malnourished. Pneumonia was diagnosed in children with fever, cough, and tachypnea, augmented by pulmonary auscultation and, when available, chest x-rays.[213,214] The guardian or the examining doctor assessed the child's feverishness without a thermometer. The HIV-positive children who presented with clinical signs of WHO disease-stage III or IV[177] (Table 4) were considered as having an advanced state of the disease.

7.3 Otorhinolaryngological examination

All study children underwent a thorough ORL examination including anterior rhinoscopy, oral and oropharyngeal cavity examination, pneumatic otoscopy, and

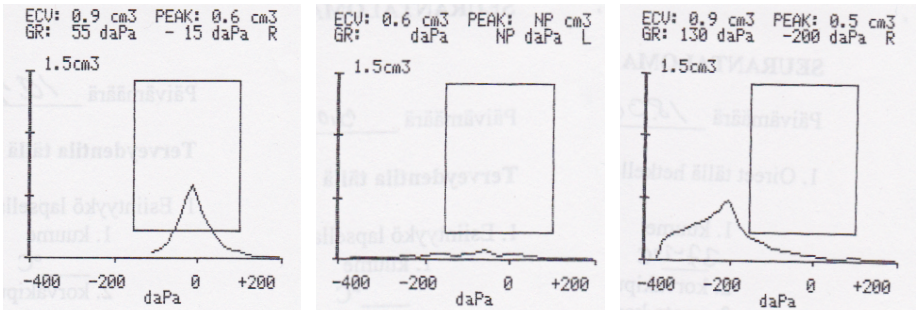
tympanometry. Any facial abnormalities were visually assessed, and the size and appearance of aurices, as well as possible existence of preaurical fistules, were recorded. In addition, the cervical area was palpated, and the size (cm) and location of palpable lymph nodes or tumors were recorded, as well as the size and consistency of the salivary glands.

The oral and oropharyngeal examination included the oral mucosa, tonsils, and the dental status. Absence of teeth was noted, as well as abnormal development, and presence of cariotic lesions in the dentition. Tonsillar size was graded on the basis of Mallampati classification 0-4 (0: tonsils fit within the tonsillar fossa; 1: tonsils 0 to 25% of the space between the pillars; 2: tonsils 25 to 50% of the space between the pillars; 3: tonsils 50 to 75% of the space between the pillars; 4: tonsils 75 to 100% of the space between the pillars).

Otologic assessment comprised pneumatic otoscopy and tympanometry. During otoscopy, the ear canal was cleansed from cerumen or suppuration with cotton swabs and alligator forceps. However, if a satisfactory view of the tympanic membrane was impossible due to tightly impacted cerumen or excessive suppuration, the case was simply defined as "cerumen impaction" or "suppuration." Diagnosis of AOM was based on detection of middle-ear fluid behind an inflamed TM, accompanied by any sign of local or general infection. Signs of fluid in the middle ear comprised visible fluid or pus behind the TM, flatness or bulging of the TM, restricted mobility of the TM. Inflamed TMs were red, yellow, or opaque. Local and general signs of infection included earache, fever, rhinorrhea, cough, and vomiting. Children with fluid in the middle ear but without inflamed TMs and evidence of an acute infection were defined as having middle-ear effusion (MEE). Suppurative discharge from the middle ear enduring for less than 2 months was also defined as AOM. Suppuration persisting for over 2 months was defined as CSOM.

Tympanometry was performed with a GSI 38 Auto Tymp tympanometer (Grason-Stadler, Inc., Eden Prairie, MN, USA). A pressure range of -400 to +200 daPa was tested with a 226-Hz probe. The tympanograms obtained were classified as A, B, and C according to the presence and location of the peak on the horizontal axis (daPa). (Figures 4 a-c) Curve gradients were not accounted for. Curves with a distinct peak at $^{-}$150 to $^{+}$100 daPa were designated as type A, which suggested normal pressure in the middle ear. Curves with a peak occurring at $^{-}$400 to $^{-}$150 daPA were designated as type C, and suggested possible negative pressure in the middle ear. Both types A and C were interpreted as indicating an intact TM without fluid in the middle ear. Peakless curves were designated as type B and considered to suggest either fluid in the middle ear, TM perforation, or cerumen impaction.

The final assessment of middle-ear status was based on both tympanometry and otoscopy. However, even if nonremovable cerumen prevented a view of the TM with an otoscope, obtaining a type A or C tympanogram was considered as ruling out TM perforation.



Figures 4a-c. Examples of tympanogram curve types A-C. ECV – external canal volume, normally in children approximately 0.6 cm3; GR – tympanogram gradient, width of graph at 50% of the maximal admittance; PEAK – the maximal (static) admittance of tympanic membrane.

a: Type A – graph with a distinct peak occurring at a pressure higher than -150 daPa (on x axis).
b: type B – flat graph with no detectable peak.
c: type C – graph with a distinct peak at a pressure less than -150 daPa.

7.4 Hearing measurements

Hearing measurements comprised BAEP threshold screening (Bera MADSEN Octavus® system v.2.001, Windows XP/2000 compatible; GN Otometrics, Taastrup, Denmark), and air-conduction pure-tone audiometry (Midimate 622 Clinical/ Diagnostic Audiometer; Madsen Electronics, Taastrup, Denmark). Click-stimulated BAEP screening was applied in all children. Pure-tone audiometry was measured without a masking tone. Audiometry was performed in children of at least 5 years of age, due to its cognitive and co-operative requirements.

In pure-tone audiometry, hearing was tested at 0.125, 0.25, 0.5, 1, 2, 4, 6, and 8 kHz (Figure 5). Starting from 1 kHz, the higher frequencies were tested first in ascending order, followed by the lower frequencies in descending order. At each frequency, the screening level was 20 dB HL. If the child failed to respond at 20 dB HL, the sound level was increased by 5 dB until a response was obtained. If the result at any frequency was assessed as unreliable, the hearing threshold was confirmed by immediate re-testing. Pure-tone averages (PTA), the mean hearing thresholds of 0.5, 1, 2, and 4 kHz, were applied in Studies II and IV, whereas in Study IV each frequency in the range 0.25 to 8 kHz was analyzed separately. The hearing thresholds were graded according to the WHO scale (Table 6).[215] While performing pure-tone audiometry, the researchers continuously assessed the surrounding noise level. In the absence of sound-proof facilities, all sounds from inside the examination room, such as air-conditioning, were quieted, and during any noise from outside the room, the screening was paused.

BAEP thresholds were measured at 40, 60, and 80 dB, (Figure 6) and the results interpreted as follows: absence of a normal response at 40 dB was considered a moderate hearing loss; absence of a response at 40 and 60 dB a severe hearing loss; and an absent response at all three levels a profound hearing loss.

Figure 5. Pure-tone audiometry measured in children aged 5 and above.

Table 6. WHO hearing impairment grades, definitions, and recommendations.[217]

Grade of hearing impairment[a]	Clinical hearing	Recommendations
Slight (26–40 dB HL)	Normal voice at a distance of 1 metre	Counselling; hearing aids recommended for children with thresholds > 30dB
Moderate (41–60 dB HL)	Raised voice at a distance of 1 metre	Hearing aids recommended
Severe (61–80 dB HL)	Some words when shouted into better ear	Hearing aids; if not available, signing and lip-reading necessary
Profound or deaf (≥81 dB HL)	Unable to understand even with shouting into ear	Hearing aids may prove useful; lip-reading, signing, additional rehabilitation essential

[a] Defined as the average pure-tone threshold of 0.5, 1, 2 and 4 kHz in the better ear.

The BAEP screening was performed without sedation and under the supervision of at least one of the researchers. Older children were seated on a chair and those younger in their guardian's lap. Attempts were made to keep each child's movements, especially eye movements, to the minimum, and the youngest children

Figure 6. Brainstem auditory-evoked potentials registered without sedation.

were calmed by breastfeeding. After the skin was first carefully dried from sweat, the electrodes were positioned on the anterior forehead and at the mastoids. In the case of excessive artefacts in the response curve, the measurement was temporarily paused until the artefact source was neutralized.

7.5 Statistical analysis

Statistical analyses of the data were performed with Statview® (v.5.0.1, SAS Institute Inc., Cary, NC, USA). Statistical correlations for nominal variables were analyzed with Pearson's chi-square and Fisher's exact tests, whichever was feasible. Medians of continuous variables were compared with Mann-Whitney's U-test. Resulting p values ≤ 0.05 were considered statistically significant.

8. RESULTS

8.1 Respiratory infections – virology and clinical profiles [I]

In this study, of 102 NPS specimens, 37 (36%) appeared positive in PCR: 27 (26%) for HRV alone, 3 (3%) for HEV alone, and 7 (7%) for both viruses. In comparison of the virus-positive and -negative children, the virus-positive children were significantly younger (36 months vs. 52 months; p=0.024). Chronic illnesses (HIV, SCD, tuberculosis) were present in 70% (26/37) of the PCR-positive vs. 63% (41/65) of the PCR-negative children (p=0.604), and malnourishment in 19% vs. 31% (p=0.218). No statistically significant differences emerged in frequencies of clinical findings among the PCR-positive vs. PCR-negative children with common colds: feverishness [12 (30%) vs. 13 (20%); p=0.161], rhinorrhea [25 (68%) vs. 35 (54%); p=0.176], cough [22 (59%) vs. 28 (43%); p=0.112], AOM [9 (24%) vs. 8 (12%); p=0.117], pneumonia [6 (16%) vs. 6 (9%); p=0.292]. Neither did other clinical findings, such as wheezing, postnasal drip, tonsillitis, hoarseness, or conjunctivitis, associate with the PCR outcome, and these findings occurred in only a few children. Five of the six picornaviral cold-associated pneumonias occurred in HIV-positive children.

Both the occurrence throughout the study of common colds and the proportion of HRV-positive specimens increased, HRV presenting in 47% (25/53) of the specimens taken in the coolest month, July, vs. 18% (9/49) in April to June combined (p=0.021; Figure 7).

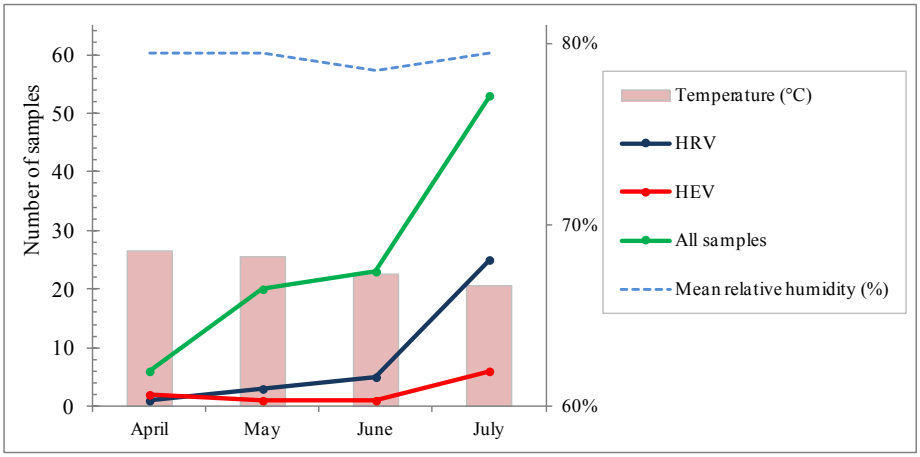


Figure 7. Emergence of human rhinovirus (HRV) and human enterovirus (HEV) in polymerase chain reaction in nasopharyngeal smears of paediatric outpatients with respiratory symptoms during 4 autumn-winter months in Luanda, Angola. The monthly mean temperatures (left vertical axis) and air humidity values (right vertical axis) are long-term averages in Luanda. (216) Adapted from: Taipale A, Pelkonen T, Roivainen M, Kaijalainen S, Bernardino L, Peltola H, Pitkäranta A. Human rhino- and enteroviruses in children with respiratory symptoms in Luanda, Angola. Accepted for publication in Paediatrics and International Child Health; with kind permission from Maney Publishing (www.maneypublishing.com/index.php/journals/pch).

8.2 Chronic suppurative otitis media – clinical and microbiological variables [II]

Background characteristics, medical history, and clinical findings were compared between 23 children with and 23 controls without CSOM (Table 7). CSOM was bilateral in 10 of the 23 (43%) cases, and its duration ranged from 2 months to 7 years (median 12 months). Six (26%) CSOM children suffered from both HIV and tuberculosis. None of the 23 children with CSOM exhibited intra- or extracranial complications.

The bacterial aetiology of CSOM appeared monomicrobial in all 18 bacterial-positive cases and comprised *Proteus* (8, 44%), *Pseudomonas* (4, 22%), *Shigella* (1, 6%), and *Haemophilus* (1, 6%) species. Four (22%) smears remained culture negative. Among these bacteria, antibiotic resistance was common against amoxicillin (64%), amoxicillin-clavulanic acid (60%), and trimethoprim-sulpha (71%), but no resistance occurred against ciprofloxacin or third-generation cephalosporins.

Among the CSOM children, slight-to-severe hearing loss (PTA or BAEP 28 to 80 dB HL) occurred in 52% (17/33) of the discharging ears, and slight hearing loss (PTA 28 to 31 dB HL) occurred in 23% (3/13) of the non-discharging ears, of which 2 had dry TM perforations and one a retracted TM. Bilateral hearing loss was present in 30% (7/23) of the CSOM children, and 6 children exhibited a PTA threshold > 30 dB HL in the better ear. Only one 8-year-old child had had attention paid previously to his hearing loss. None of the control children had bilateral hearing loss.

8.3 Sickle-cell disease-associated otorhinolaryngological pathology [III]

This study comprised 61 children with and 61 controls without SCD. Both groups included 36 (59%) boys, and the median ages of the children were 4.9 years (range 0.7-15.7) in the SCD and 4.8 (range 0.8-13.7) in the control group. Among the SCD children, 41 (67%) took regular medication (mainly folic acid, paracetamol, and ibuprofen), and 12 (19%) had received blood transfusions. For no SCD children were penicillin prophylaxis and pneumococcal vaccines available.

Table 8 shows that no significant differences occurred among the SCD vs. control children in ORL findings, including middle-ear and TM pathology.

In pure-tone audiometry screening, failure to hear a 25 dB HL stimulus at one or more frequencies was the case for 15 (60%) SCD- and 14 (50%; p=0.465) control children. Bilateral hearing loss occurred in 9 (36%) SCD and 3 (11%; p=0.047) control children (Figure 8). These hearing losses were of slight degree (30-40 dB HL), except for solitary losses of moderate degree in two SCD and two control children. None appeared to need a hearing aid. In SCD vs. control children, neither the average hearing thresholds nor the failure rates differed significantly at any frequency.

Table 7. Selected background features and clinical findings of children with (N=23) and without (N=23) chronic suppurative otitis media (CSOM) in Luanda, Angola. Comparisons by Pearson's chi-square and Fisher's exact tests. Adapted from: Taipale A, Pelkonen T, Taipale M, Bernardino L, Peltola H, Pitkäranta A. Chronic suppurative otitis media in children of Luanda, Angola. 2011; 100: 84-88. © 2011 The Authors / *Acta Paediatrica* © 2011 Foundation Acta Paediatrica.

	CSOM children n (%)	Controls n (%)	p value
Background features			
Female	11 (48)	11 (48)	
Median age (years); range	4.3; 1.1–11.5	4.5; 0.8–12.5	
Number of children in the household; range	2; 1–7	3; 1–7	0.583
Running water at home	8 (35)	6 (26)	0.749
Electricity[a] at home	16 (70)	16 (70)	>0.999
Attendance at national vaccination program	20 (87)	17 (74)	0.265
Median duration of breastfeeding, months (range)	18 (1-24)	18 (3-37)	0.114
Earlier episodes of otorrhoea	23 (100)	3 (14)	< 0.001
Ear pain, earlier or currently	23 (100)	0	< 0.001
Clinical findings			
Acute pulmonary infection	9 (39)	1 (4)	0.010
Cervical lymphadenopathy > 1cm	15 (65)	5 (22)	0.007
Tuberculosis	8/22 (36)	0	0.002
HIV positivity	14/22 (64)	0	< 0.001
Malnutrition	8 (35)	2 (9)	0.071

[a] During several hours 1-2 times daily through public electric transmission

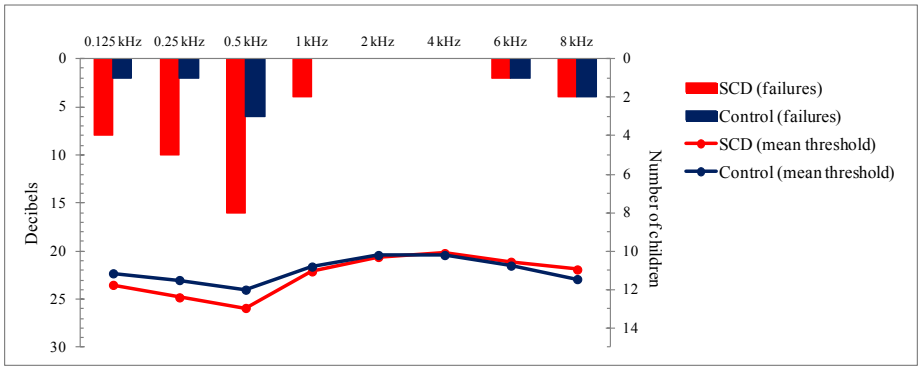


Figure 8. Mean hearing thresholds in decibels (lines; left vertical axis), and the numbers of children failing to hear a 25 dB stimulus with their better ears (columns; right vertical axis), among 25 children with sickle-cell disease (SCD) and 28 controls in Luanda, Angola. Adapted from: Taipale A, Pelkonen T, Bernardino L, Peltola H, Pitkäranta A. Hearing loss in Angolan children with sickle-cell disease. Pediatrics International 2012; 54: 854-857. © The Authors. Pediatrics International © Japan Pediatric Society.

Table 8. Otorhinolaryngological profiles in 61 children with sickle-cell disease (SCD), their 61 controls, and 78 children with human immunodeficiency virus (HIV) and their 78 controls in Luanda, Angola (compared by Pearson's chi-square and Fisher's exact tests).

	SCD			HIV		
	Patients n (%)	Controls n (%)	p value	Patients n (%)	Controls n (%)	p value
History						
Otalgia	25 (41)	31 (51)	0.276	44 (56)	27 (35)	0.006
Otorrhea episodes	6 (10)	11 (18)	0.191	34 (44)	17 (22)	0.004
Hearing complaints	5 (8)	9 (15)	0.395	6 (8)	9 (12)	0.415
Speech delay	1 (2)	2 (3)	>0.999	2 (3)	1 (1)	>0.999
Current findings						
Respiratory infection	29 (48)	22 (36)	0.286	55 (71)	25 (32)	<0.001
Dermal lesions[a]	3 (5)	3 (5)	>0.999	25 (32)	4 (5)	<0.001
Oral candidiasis	1 (2)	3 (5)	0.619	9 (12)	5 (6)	0.402
Dental caries	26 (43)	23 (38)	0.580	44 (56)	25 (32)	<0.001
Tonsillar hypertrophy[b]	10 (16)	6 (10)	0.334	1 (1)	6 (8)	0.063
Salivary gland hypertrophy	2 (3)	0	0.496	2 (3)	0	0.497
Cervical lymph nodes[c]	11 (18)	4 (6)	0.096	35 (45)	8 (10)	<0.001
Acute otitis media	2 (3)	4 (6)	0.680	8 (10)	9 (12)	>0.999
Chronic suppurative otitis media	0	2 (3)	0.496	21 (27)[d]	3 (4)	<0.001
Middle-ear effusion	1 (2)	1 (2)	>0.999	0	0	>0.999
Dry tympanic membrane perforation	1 (2)	53 (48)	>0.999	7 (9)	1 (1)	0.063
Cerumen impaction	27 (44)	30 (49)	0.586	26 (33)	34 (44)	0.249

[a] Maculopapular eczema, folliculitis, molluscum contagiosum
[b] At least 50% of the space between pillars
[c] At least 1 cm
[d] Eight (38%) cases bilateral

Of the 15 SCD children (6 to 16 years old) who exhibited uni- or bilateral hearing loss, 2 children (3 ears) had cerumen impaction; of the rest, none manifested AOM, MEE, or TM perforations. Two children had previously noticed their own hearing loss.

8.4 Human immunodeficiency virus-associated otorhinolaryngological pathology [IV]

This study compared ORL status in 78 HIV-positive children and 78 controls. The median age in the HIV vs. control group was 4.3 years (range 0.8-14.8) vs. 4.1 years (range 0.8-13.7), and both groups included 36 (46%) boys. Of the HIV-positive children, 41 (53%) showed clinical signs of advanced or severe disease according to WHO criteria; 36 (46%) received antiretroviral treatment, 61 (78%) antibiotics (mainly sulfa-methoxazole), and 5 (6%) anti-tuberculosis treatment. Concurrent or previous diagnosis of tuberculosis occurred in 22/77 (29%) HIV-positive children vs. 2/77 (3%; $p<0.001$) controls, current diagnosis of pneumonia in 17 (22%) HIV-positive vs. 2 (3%; $p<0.001$) controls, and current hepatomegaly in 8 (10%) HIV-positive vs. zero controls ($p=0.007$).

Major differences emerged in the ORL findings among the HIV-positive vs. control children, as described in Table 8. None of the clinical variables inside the HIV group were associated with these children's antiretroviral treatment status.

Hearing screening by BAEP was performed in all study children, and 29 HIV-positive and 31 control children also underwent audiometry. No data on hearing could be obtained in 2 young control children (3 ears), in whom BAEP screening failed, and pure-tone audiometry was nonfeasible. After combining the results from these two screening methods, 20 (26%) HIV-positive and 12 (15%) control children exhibited slight-to-profound hearing loss. Bilateral hearing loss was present in 10 (13%) HIV-positive children vs. one (1%; $p=0.009$) control child. In the HIV-positive children, otoscopy of the 30 hearing-impaired ears revealed CSOM (12; 40%), cerumen impaction (7; 23%), dry TM perforation (5; 17%), intact, mobile TM (4; 13%), and retracted TM (2; 7%). Hearing defects among the HIV-negative children (13 ears) related to cerumen (5; 38%), to unknown factors (5; 38%), to AOM (2; 15%), and to CSOM (1; 8%).

9. DISCUSSION

9.1 The role of picornaviruses in tropical respiratory infections

In the present study, screening picornaviruses in children with respiratory symptoms from various outpatient clinics, the incidence of respiratory symptoms increased in the cool season. Picornaviruses occurred at young ages, a median 3 years, with symptoms of fever, rhinorrhea, and cough. The frequencies of these symptoms, as well as otitis media (discussed later in detail) and pneumonia, showed no alteration related to the children's picornavirus positivity or underlying chronic conditions. Furthermore, neither HIV infection, SCD, nor malnutrition associated with picornavirus positivity.

In this sample, proportions of children with HIV infection and SCD were highly overrepresented in relation to those of the general population. This is largely due to the high contribution of these chronic illnesses to health care: In the mid-2000's, systematic HIV testing of patients in the Paediatric Hospital of Luanda revealed roughly one-fifth to be HIV positive.[216] Notably, the present study's SCD and HIV children exhibited no higher frequencies of acute respiratory symptoms than in control children.

Here, prevalence rates for HRV and HEV positivity were consistent with those in other recent studies conducted among outpatients in subtropical or tropical countries.[48,49,51,217,218] Strictly speaking, the present study could verify no causality between viral shedding and clinical infection, as that would have demanded serial NPS sampling, and obtaining and processing multiple samples from the same individuals would have posed excessive economic and logistical challenges regarding the study context.

Our findings suggest that HRV transmission may increase in the tropics at decreasing temperatures: HRV caused nearly half of all RTI cases in the coolest month of July. However, the limited study period (April to July), as well as the relatively small sample size, restricts further speculation on picornavirus seasonality. None of the larger-scale studies from tropical regions reported a clear seasonality for picornaviruses: association with weather parameters went either undetectable[48,49] or unanalyzed.[217] A cohort study from tropical Brazil perceived a peak in HRV incidence during the local autumn, however.[50]

Knowledge of the aetiology and epidemiology of RTIs is recognized to be of major importance in developing countries where these diseases carry high morbidity and mortality.[219] Until recently, HRV has been considered a rather harmless virus responsible mainly for mild RTI symptoms, and it has gained little attention in research in developing countries. Because up-to-date evidence links HRV not only

to lower RTIs,[21,22,33,48,53,220-222] but also to invasive pneumococcal diseases,[223,224] and even to meningitis,[39] research on picornavirus infections in developing countries becomes more relevant. In addition, the underlying nutritional or immune deficiencies and their impact on the clinical picture and outcomes of common cold deserve more attention.

Because the multiplicity of HRV and HEV types hampers effective vaccine development, restricting the transmission of these viruses remains the major preventive measure.[219] Further research is therefore needed on the epidemiological and clinical aspects of HRV and HEV infections in developing countries.

9.2 Middle-ear pathology

9.2.1 OTITIS MEDIA AS A COMPLICATION OF RESPIRATORY INFECTION

AOM occurred in one-quarter of the children with picornavirus infections. More than half of all AOM children who underwent NPS sampling actually yielded picornavirus, but this association lacked statistical significance. Should any real association between AOM and picornaviruses among all RTI-causative agents have existed, demonstrating it would probably have demanded a considerably larger sample size, because of the high overall frequency of AOM during viral RTIs. As such a high proportion of children with RTI will develop AOM anyway, picornaviruses may not, from an epidemiological point of view, outweigh other aetiopathological viral agents of AOM in their middle-ear invasiveness but simply by their higher frequency. Actually, the proportions of AOM and picornaviral RTI that we obtained agree with findings from temperate climates, where picornavirus RTIs lead to AOM in approximately one out of three cases,[18] and were present in up to half the AOM children.[41,42,57,58]

This study suggests a marked involvement of picornaviruses in the AOM complication of tropical common colds. The extent of this involvement remains a subject for further research in the quest for more accurate characterization of AOM – the major pathway to CSOM in areas of a high burden of chronic otitis media.

9.2.2 CHRONIC SUPPURATIVE OTITIS MEDIA

We evaluated CSOM in children with otorrhoea of up to 7 years' duration. Studies from developing countries verify that children may suffer from extremely longstanding otorrhoea, because treatment is delayed or lacking.[70,81,111,112,225] The median age of our CSOM children was 4 years. Several earlier studies corroborate the young age of CSOM patients.[65,73,112,115,116,225] As for ours, more than 40% had bilateral disease, similar to common findings in childhood CSOM in developing countries.[70,73,80,82,112,225] All of these features long duration, early onset, and a tendency toward bilaterality

– underline the importance of prompt treatment of CSOM during the child's critical age of linguistic and cognitive development.

The present study excluded children whose otorrhoea had persisted for less than 2 months. Worldwide, the definition of CSOM is not uniform, and durations of at least 2 weeks to 3 months have been suggested as defining middle-ear discharge as being chronic. In the Paediatric Hospital of Luanda, the vast majority of children with otorrhea had at least several months' history of the disease, and thus the data obtained by applying the cut-off limit of 2 months represented the actual patient material well.

Our most frequent bacterial causative agents were *Proteus* and *Pseudomonas* species. This is indeed typical of developing countries,[65,66,69-71,73,112] except that *Staphylococcus aureus* was absent from our patients. However, our sample size was limited, and therefore coincidence most probably explains this non-emergence of *Staphylococci*. Additionally, pus sampling from the ear canal, instead of as an aspirate directly from the middle ear, may have led to a higher risk of contamination. On the other hand, one study from India detected no difference between these two sampling methods, and so our results may be considered rather reliable.[226]

The high frequency of opportunistic Gram-negative rods reflects the poor general state of health and the prolonged disease process in our CSOM children and thus points to their condition's severity.

Mycobacterium diagnostics, had it been performed, would have provided valuable additional information because pulmonary tuberculosis occurred in more than one-third of our CSOM children. Considering this high tuberculosis prevalence, as well as the severity of hearing loss in some patients, some of our children may actually have suffered from aural tuberculosis. In general, tuberculous otorrhea is regarded as a rare disease offering diagnostic challenges.[227,228] In future, a study on mycobacterial CSOM among HIV-positive children in resource-poor settings would be of value.

No intra- or extracranial complications of CSOM were encountered. This was rather expected, as the CSOM children were outpatients visiting the clinic for other reasons. However, because complications of CSOM continue to cause morbidity and mortality in children of sub-Saharan Africa, the subject requires research in the settings where such cases more likely will emerge, i.e. in ORL clinics or during acute health care. For the same reason – CSOM-induced adverse outcomes – general research on childhood CSOM in developing countries needs to continue.

9.2.3 MIDDLE-EAR PATHOLOGY IN SICKLE-CELL DISEASE

Regarding the impact of SCD on childhood middle-ear infections in Luanda, unexpectedly, SCD children only occasionally exhibited middle-ear pathology, despite their lack of penicillin prophylaxis and pneumococcal vaccines. Few reports

concerning otitis media in SCD children exist: 3 hearing-screening studies (2 from the USA, one from Nigeria), reported MEE in up to 27.5%.[139,142,159] Of these, one US study perceived an increased prevalence of middle-ear pathology in SCD vs. control children,[142] while in Nigeria the rates appeared equal.[139] In addition, a retrospective survey from the USA detected fewer tympanostomy-tube insertions in SCD children than in a historical SCD-free cohort.[229] Recurrent, persistent, and complicated episodes of otitis media are, however, characteristic of children without haemoglobinopathies but with antibody deficiencies.[165,230,231] SCD children with their weakened splenic function might therefore be expected to show a tendency towards acute and chronic otitis and its severe suppurative complications, but actual evidence on the subject remains small and controversial.

Possible explanations for the absence of middle-ear pathology among our SCD children include a better clinical balance in this group, when compared to the worse-off majority not followed up at the SCD clinic; survival of children with a milder form of SCD (the most severe cases leading to early mortality); and, finally, mere coincidence. Concerning this study, we must also emphasize the possibility of missing small amounts of MEE in young children with suboptimal otoscopic views due to impacted cerumen in the ear canal. Future studies should include a larger and wider sample of SCD children and controls, as well as follow-up.

Some studies suggest only a low proportion of *Streptococcus pneumoniae* in bacteraemia of SCD children in malaria-endemic regions; this has raised the question whether malaria-induced splenomegaly could partially preserve splenic function in these children.[232,233] Malaria may thus be one of the environmental factors modifying the course of SCD. A follow-up study is required to verify the impact of SCD on the incidence and course of childhood otitis media in low-resource, malaria-endemic regions where both SCD and otitis-related deaths occur.

9.2.4 THE BURDEN OF OTITIS MEDIA IN HUMAN IMMUNODEFICIENCY VIRUS–POSITIVE CHILDREN

In all HIV-positive children of the present study, CSOM was present in more than one-quarter and dry perforations in one-tenth. Our small and hospital-based samples may have been biased, and our CSOM group probably exaggerates the true contribution of HIV in CSOM epidemiology. However, several studies confirm the high burden of chronic otitis in HIV-positive children,[201-204] and reports also confirm the occurrence of systemic complications of otitis media among them.[187,201]

No previous reports were available of incidence rates or wider statistics concerning the role of HIV in otitis media and its complications in developing countries. In Brazilian HIV-positive children, management of HIV alleviated the burden of their otitis media.[207] The impact of the HIV epidemic on the course and consequen-

ces of CSOM is probably considerable in regions of high HIV prevalence, and thus deserves future research.

9.3 Otorhinolaryngological profiles in different patient groups

Of every ten of our children without clinical suspicion of HIV, seven exhibited ORL pathology, making ORL pathology apparently highly frequent. The most typical ORL symptoms and diseases were respiratory infections, lymph-node enlargement, ear pain, acute or chronic otitis media, and oral thrush. In children of developed countries, RTIs[234] and AOM[235] are also usual. However, chronic otitis and oral candidiasis are ORL presentations typical of children who experience poor living conditions and nutrition and hygiene deficiencies.

In our children, cerumen impaction was frequent. The children or their caregivers were only rarely familiar with the meaning or normal function of ear wax. Indeed, cerumen impaction is common in children of resource-poor countries and may even lead to clinically significant hearing loss.[236-239] In children with already compromised hearing due to other causes, even slight additional cerumen-induced hearing loss may significantly worsen hearing ability. In general, not many studies concern the adverse effect of excessive cerumen on hearing. Among some adults in the UK, one-third benefited from removal of impacted cerumen, and even though the procedure improved hearing levels by an average of only 5 dB HL, some individuals' hearing levels improved by up to 36 dB HL.[240]

In populations with high prevalences of TM perforations, limited knowledge of anatomy of the ear, and poor availability of clean water or cerumenolytic agents, removal of cerumen should occur in health care centres with trained personnel, manual removal without irrigation probably being the most feasible method.

In this study, of every ten HIV-positive children, no less than nine exhibited ORL pathology. This is consistent with reports that also uniformly suggest substantial ORL involvement in paediatric HIV infection.[184-186,188] Compared to our controls, the present study's HIV-positive children presented a higher number of pathologic findings with a more severe clinical picture. They had marked cervical lymphadenopathy and rampant caries more frequently, but so did some control children, as well. In the head and neck, bilateral CSOM and widespread eczema appeared particularly strong indicators of HIV positivity. Compared to earlier reports, we detected HIV-associated oral candidiasis somewhat less and parotid enlargement much less frequently.[184-186,188] On the whole, our findings suggest that, as part of their medical follow-up, HIV-positive children should routinely undergo an ORL examination.

In contrast to the HIV patients, the present study was unable to demonstrate any significant elevations in the frequencies of ORL findings in the SCD children. No other comparative studies on the subject have emerged. The present study's SCD

children also exhibited none of the reported ORL complications of SCD, such as deep infections, osteomyelitis, or marked tonsillar hypertrophy.[166,170] This suggests that such findings are fairly unusual even in developing countries. However, because ORL complications of SCD do exist in case reports, defining their occurrence would be interesting in resource-poor settings where the majority of the patients live.

9.4 Hearing Loss

9.4.1 HEARING LOSS DUE TO CHRONIC SUPPURATIVE OTITIS MEDIA

The present study detected at least slight hearing loss in half the CSOM ears and bilateral hearing loss in nearly one-third of CSOM children. Only one of these cases had been noticed by the family. These characteristics are consistent with findings in developing countries that of every 10 CSOM children, hearing loss occurs in 5 to 9.[79-82,114-116] Data also show that both otorrhoea and hearing loss in children in resource-poor settings remain widely neglected.[73,112,241] Unfortunately, the onset of CSOM during infancy may pose a higher risk for hearing loss.[116] Chronic otitis media occurring at school age may cause hearing loss sufficiently severe to endanger scholastic performance.[82,117,239] Therefore, in resource-poor communities, more precise recognition of a child's ear discharge as a warning sign is warranted in order to fully utilize existing resources for prevention of and rehabilitation of hearing loss.

Information should be targeted to communal workers serving children and families at clinics and schools, as well as to primary health care professionals. As CSOM children typically suffer from slight-to-moderate hearing loss, they would, even without access to hearing aids, be substantially supported by simple rehabilitative measures such as having seats in the front of the classroom, being addressed in a loud and clear voice, and making lip-reading more possible – instead of their being erroneously regarded as lazy or unintelligent.

9.4.2 HEARING LOSS IN SICKLE-CELL DISEASE

In audiometric evaluation of our SCD children, screening failures occurred in three-fifths of them and bilateral hearing loss in more than one-third. However, only bilateral hearing loss was significantly associated with SCD. The absence of evidence of middle-ear pathology suggests that the hearing impairment may have originated mainly from the inner ear. Of the 15 hearing-impaired SCD children, however, in 2 (3 ears), cerumen impaction prevented reliable otoscopic and tympanometric evaluation and may have been the actual reason for compromised hearing. Only 2 hearing-loss cases had previously been detectable. None presented with a PTA threshold >30 dB HL in the better ear, the current WHO standard for considering a hearing aid.[4] The unexpectedly high hearing-loss rate among the present study's

control children may have been coincidental, or a result of higher participation of families of children with ear- or hearing problems.

We detected a relatively high rate of hearing loss compared to that for previous researchers, particularly if losses attributable to middle-ear pathology in the earlier data are excluded. Notably, half the hearing-impaired children were only 5 to 8 years of age, younger than in most studies, which predominantly examined older children.[139,142-145] We thus highlight the risk for hearing loss in SCD that occurs even as early as at ages 5 to 6. In contrast to some reports,[142,145] we found no severe impairment, which suggests that severe hearing loss occurs in children only rarely. However, even slight or fluctuating hearing loss may adversely affect a child's speech development.[242]

With improved management, more SCD children will survive in sub-Saharan Africa beyond early childhood.[119] In these survivors, screening for hearing problems should be implemented in their routine care even at preschool age.

9.4.3 HEARING PROBLEMS IN HUMAN IMMUNODEFICIENCY VIRUS-POSITIVE CHILDREN

Among the HIV-positive children, some degree of hearing loss occurred in one-quarter, and half of those suffered bilateral loss. More than half the hearing loss occurred in ears with TM perforations, the majority of which were actively discharging. This is consistent with data in other studies from developing countries, showing hearing loss in 23 to 39%.[191-194] These studies also frequently detected middle-ear pathology behind the impairment. In resource-rich settings, the proportion of otitis media in HIV-associated hearing loss may be lower, but risk for hearing loss remains elevated.[243]

The present study confirms and underlines the importance of recognizing hearing loss and treating otitis media promptly in HIV-positive children in developing countries, as well as the importance of screening their hearing on a regular basis.

9.5 Practical and ethical viewpoints

Our study was conducted in a paediatric hospital in tropical Angola, where few data exist on childhood ORL problems. The study children were simply recruited among the hospital's outpatients, which provides both strengths and limitations for the study. Obviously, the samples were highly biased compared to the general population: In addition to chronic underlying diseases, higher socioeconomic classes may have been overrepresented among the hospital's outpatient population, as even public transportation to the hospital may have been too expensive for the poorest families. Moreover, factors such as higher education, positive family history, or

suspicion of existing ORL problems may have furthered the guardian's decision to participate in the study. However, in developing countries, children ask for medical care due to ORL problems only rarely. These problems are and should therefore be screened for and detected during visits for other reasons; this underlines the clinical relevance of the present findings.

In resource-poor Angola, basic ORL services, even ones such as otoscopic examination, are available only rarely. Soundproof facilities for hearing screening and ear-suction devices for cleansing the ear canal were nonexistent, and electricity was undependable. These circumstances contribute to this study's limitations: pure-tone thresholds obtained, especially in the lowest frequencies, may be somewhat higher than in reality, and in some cases (especially in young children) not all cerumen could be removed from the ear canal. BAEP screening proved challenging due to excessive sweating when the air-conditioner was broken or lacked electricity. Such circumstances represent, however, the usual settings in health care and research practiced in resource-poor countries.

Pure-tone thresholds were measured without masking the contralateral ear. Due to transaural bone conductibility, this may have resulted in erroneously low pure-tone thresholds in the worse-hearing ears in children whose pure-tone thresholds differed by more than 50 dB HL. In addition, as no bone-conduction thresholds were measured, a distinction between conductive and sensorineural hearing loss was impossible. Furthermore, because in cochlear hearing loss the correlation between hearing thresholds obtained by click-evoked BAEP vs. pure-tone audiometry is strong only at 2 to 4 kHz and remains weaker at lower frequencies,[244] the ability of our BAEP measurements to detect such hearing loss cases was probably suboptimal.

To be ethical, research in a resource-poor environment should have a clear purpose,[245,246] which, in the present study, was to collect epidemiological and clinical data on childhood ORL diseases affecting millions of children worldwide. In addition, making medically purposeful research ethically sustainable requires ethical evaluation by local professionals, as well as consent from the study attendants. The present study underwent ethical consideration in the Paediatric Hospital of Luanda.[245,246] Because of limited literacy among Angolans, however, study information and consent toparticipate was handled orally. The majority of the guardians were familiar with neither the usual childhood ORL diseases nor the concept of medical research.

According to Ruth Macklin, these examples represent a true dilemma for research among resource-poor communities: should ethical guidelines arise from pragmatism or idealism; how can one create guidelines that are, as Macklin noted, "usefully prescriptive without being hopelessly aspirational"? In a culture which knows no basic concepts of medicine or medical research, how to adapt research procedures, such as obtaining informed consent, to the environment in such a way that the consent obtained becomes genuine? Macklin states that research concerning a major health issue in the target population can be ethically justified, if no

possibility exists for conducting it in another population with more potential for understanding its content.[247] In the team of our present study, the local professionals plus one member with long-term local experience lowered these cultural barriers.

In addition to the wider advantages for future patients, short-term research may locally provide for the participants "ancillary care" not directly related to the study protocol.[245,246] Here, ancillary care (e.g. medical prescriptions) was provided when needed, but did not serve as a "decoy" in recruitment, although sometimes families with known ear complaints appeared more willing to participate in hope of free treatment. Leaving such families virtually without access to any treatment would have been the most unethical option. Unfortunately, in the absence of any local ORL service network, no follow-up for these children was available.

In Luanda, resources for rehabilitation of the hearing impaired are poor. Some study participants could be advised to contact a local school for the hearing impaired, but hearing-aid rehabilitation was unavailable. However, in resource-poor communities, even recognizing the hearing impairment would help the child's family and teachers to support the child in daily communication.[236]

ORL diseases and hearing loss in children of developing countries are largely neglected areas in medical research, with more data required. Worldwide, 90% of the need for hearing aids exists in developing countries, where only less than one in 40 will receive the required services.[4]

In resource-poor countries, vast, unselective screening programs requiring expensive equipment and training of personnel may be unfeasible in the absence of any functioning referral and rehabilitation system. However, not to screen at all would inevitably lead to permanent disability for hundreds of thousands of hearing-handicapped children.[248] More regional data on childhood hearing loss is therefore essential for targeted screening.[7,14,249] Questionnaires are one tool proposed for identifying children in need of hearing screening. The present findings suggest that, should such questionnaires be applied, they should include the child's HIV and possibly also SCD status. Additional simple methods for hearing assessment in primary health care, such as whispering tests,[17,250] also need research and validation in developing countries.

The child's optimal cognitive and social development relies on adequate hearing, and underprivileged communities need the full potential of their children, the builders of the future. The lifetime cost of childhood hearing loss has been estimated to exceed that of the cases with later onset.[7] In resource-poor settings, therefore, prevention and rehabilitation of hearing loss generally deserve higher priority, and the highest priority for these services should belong to children.

10. SUMMARY AND CONCLUSIONS

A massive shortage of ORL services, education, and research exists in developing countries where the resource-poor environment poses risk factors for chronic and recurrent ORL diseases. The burden of ORL diseases remains therefore high in these areas among children. In sub-Saharan Africa, one ORL expert may serve millions of people, and awareness of ORL diseases in primary care remains limited. Hearing loss and its impact on society are under-recognized, and only one in 40 in need of hearing rehabilitation receives the needed device. In the children of Africa, the HIV epidemic and high prevalence of sickle-cell disease and tuberculosis further affect the pathology and conditions related to the ORL area.

The present study was conducted in the Paediatric Hospital of Luanda, Angola, to collect data on ORL pathology and hearing in children of various outpatient clinics, and particularly to define the occurrence of HRV and HEV during the cool season, the clinical and microbiological characteristics of childhood CSOM, and the HIV- and SCD-associated ORL findings and hearing loss. Background features and medical history came from the children's guardians, and the children underwent general physical and ORL examination, plus hearing screening by BAEP and pure-tone audiometry.

Picornaviruses were evident in more than one-third of common-cold patients. Common-cold symptoms, as well as the proportion of rhinovirus as the causative agent, increased as the temperature fell. The virus-positive children were significantly younger than the virus-negative, and AOM complicated the picornaviral RTIs in about one-quarter. In resource-poor living conditions, chronic ear discharge typically harbored gram-negative rods, and in the setting of outpatient clinics, the vast majority of the patients suffered from HIV or tuberculosis; approximately one-third had slight-to-severe bilateral hearing loss. SCD children also presented with an ascending risk for hearing loss, mainly in the low and middle frequencies; these losses appeared slight, and the children exhibited no signs of the alleged susceptibility to otitis media. Abnormal ORL findings such as cervical lymphadenopathy, RTI, and otitis media were common in all children, but the children with HIV infection appeared to be major carriers of the burden of ORL diseases. Moreover, hearing loss was associated with HIV and mainly coincided with middle-ear pathology; the present study did not, however, rule out concomitant inner-ear pathology.

Several conclusions can be drawn. This work suggests the marked involvement in sub-Saharan Africa of picornaviruses, particularly HRV, in AOM epidemiology. In order to prevent more severe forms and bacterial complications of viral RTIs, more complete understanding is needed for the epidemiology and clinical consequences of picornaviruses in tropical areas with low resources and high co-morbidity.

In developing countries, a child may grow up with chronic ear discharge and hearing loss without the guardians or even medical professionals recognizing the condition. Especially in areas of the HIV epidemic, the prevalence and epidemiology of CSOM require updating.

SCD may lead to hearing loss, and children aged 5 to 6 should already be undergoing audiometric screening. However, the incidence and clinical picture of otitis media remains unclear among children with SCD in sub-Saharan Africa, and deserves a prospective follow-up study.

Ear discharge, extensive rashes, and persistent cervical lymphadenopathy may be signs of underlying HIV infections, and follow-up of HIV-infected children should include thorough ORL status, hearing screening, and prompt treatment of middle-ear infections.

Focusing attention on children's ears and hearing could substantially benefit many underprivileged communities. Both from the perspective of humanity and economy, these children are too valuable a resource to be wasted through preventable disability and suffering.

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Hyvinkää, August 2013

Anni Taipale

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APPENDIX

Study forms translated from Portuguese originals.

HISTORY AND PHYSICAL EXAMINATION

page 1

Name: Residence:

Study number_____

Date of birth: Age: Telephone:

HOSPITAL PEDIÁTRICO DAVID BERNARDINO

Gender Female ❑

Male ❑

Date of admission:_____/_____/_____

Weight :_____Kg Upper middle arm circumference:_____ cm Height_____cm

Head circumference:_____cm Blood pressure_____/_____mmHg, P_____

HISTORY

	YES	NO	Unknown
HOUSEHOLD SIZE _____persons, _____adults,_____children			
RUNNING WATER AT HOME_____	❑	❑	❑
ELECTRICITY AT HOME_____	❑	❑	❑
BREASTFEEDING Until_____months	❑	❑	❑
SOLID FOOD Introduced at_____months	❑	❑	❑
ATTENDANCE AT VACCINATION PROGRAM_____	❑	❑	❑
ANTES SAUDÁVEL_____	❑	❑	❑
CHRONIC DISEASES_____	❑	❑	❑
PREVIOUS DISEASES_____			
MALARIA_____	❑	❑	❑
TYPHOID FEVER_____	❑	❑	❑
TUBERCULOSIS_____	❑	❑	❑
HIV / AIDS_____	❑	❑	❑
PREVIOUS ADMISSIONS_____	❑	❑	❑
MEDICATION; WHICH?_____	❑	❑	❑
ANTIBIOTIC; WHICH?_____	❑	❑	❑
QUININE BEFORE_____	❑	❑	❑
SURGERY BEFORE_____	❑	❑	❑
REASON FOR CONSULTATION_____			
EARACHE; Specify_____	❑	❑	❑
FEELS THAT HEARS WELL; Specify_____	❑	❑	❑
HEARING PROBLEMS IN COMPARISON WITH OTHER CHILDREN	❑	❑	❑
HEARING DIFFICULTIES IN THE FAMILY_____	❑	❑	❑
Specify_____			

PHYSICAL EXAMINATION

	YES	NO	Unknown
GOOD GENERAL CONDITION:_____	❑	❑	❑
SIGNS OF MALNUTRITION:_____	❑	❑	❑
	❑ slight	❑ mod.	❑ grave
FOCUS OF INFECTION Specify_____	❑	❑	❑

HAEMOGLOBIN _____g/dl

OTHER INFORMATION_____

page 2

OTORHINOLARYNGOLOGICAL STATUS **YES** **NO** **Unknown**

FACE NORMAL ❑ ❑ ❑

Specify: _____

AURICLES NORMAL ❑ ❑ ❑

Specify: _____

NOSE : Direct / Deviated _____

NASAL SEPTUM: Direct / Deviated _____

RIGHT NOSTRIL: Rhinorrhea / Crust / Polyp / Normal _____

LEFT NOSTRIL: Rhinorrhea / Crust / Polyp / Normal _____

LIPS-GINGIVAS-PALATE NORMAL _____ ❑ ❑ ❑

Specify: cleft palate_____

DENTITION NORMAL_____ ❑ ❑ ❑

TEETH: Healthy / Caries / Obturated / Missing_____

ORAL MUCOSA NORMAL _____ ❑ ❑ ❑

Specify: Vesicles / Leukoplakia / Erythroplakia / Candidiasis_____

TONSILS NORMAL_____ ❑ ❑ ❑

Right tonsil: 0, 1, 2, 3, 4+ _____ Left tonsil: 0, 1, 2, 3, 4+ _____

UVULA NORMAL_____ ❑ ❑ ❑

TONGUE: Normal / Big _____

LINGUAL FRENULUM: Normal / Short _____

SPEECH NORMAL_____ ❑ ❑ ❑

Specify: Not comprehensible / Stuttering / Pronunciation difficulties _____

VOICE: Clear / Hoarse _____

STRIDOR: Reason?_____ ❑ ❑ ❑

EYE MOVEMENTS NORMAL_____ ❑ ❑ ❑

Specify: Right / Left / Strabismus / Nystagmus_____

OTHER INFORMATION:_____

DOCTOR'S SIGNATURE_____

OTORHINOLARYNGOLOGICAL STATUS

	YES	NO	UNKNOWN
PALPATION OF NECK NORMAL_____	❑	❑	❑
Specify: Tumor / Cyst _____			
LYMPHADENOPATHY; Lymph nodes, size_____cm	❑	❑	❑
FISTULES_____	❑	❑	❑
Specify: Right / Left / Preauricular / Type I / Type II / Secretion			
SALIVARY GLANDS NORMAL_____	❑	❑	❑

PAROTID	Right:	Left:
SUBMANDIBULAR	Right:	Left:
SUBLINGUAL	Right:	Left:

FACIAL NERVE NORMAL_____	❑	❑	❑

FROWNING	Dto:	Esq:
CLOSING EYES	Dto:	Esq:
SMILING	Dto:	Esq:

WEBER_____

	RIGHT EAR	LEFT EAR
RINNE		
OTORRHEA		
OTOSCOPY: Auditory canal		
OTOSCOPY: Tympanic membrane		
Mobility of tympanic membrane		
TYMPANOMETRY		
HEARING TESTS		
DATA		
NAME OF EXAMINATOR		
BAEP COMPLETED		
HEARING THRESHOLD, dB		
OAE COMPLETED		

OTHER INFORMATION_____